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Commercially Available Enzyme-Linked Immunosorbent Assay and Polymerase Chain Reaction Tests for Detection of Feline Immunodeficiency Virus Infection

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Background: Feline immunodeficiency virus (FIV) infection is an important cause of disease of cats worldwide. Initial screening is commonly performed by commercially available point-of-care (POC) ELISA tests. Confirmatory testing for positive POC test results is recommended. Polymerase chain reaction (PCR) tests for FIV are commonly used additional testing methods; however, reported measures of diagnostic accuracy vary widely between PCR tests, making interpretation of results difficult.

Hypothesis/Objective: There is very good agreement between results of a commercially available PCR test and a POC ELISA test for FIV for specimens collected from owned and shelter-housed cats.

Animals: Blood samples from 168 cats from 2 adoption guarantee shelters, an FIV Sanctuary, and 64 private homes were used.

Methods: This was a prospective study. Whole blood samples were collected in K_2-EDTA, divided, and submitted for PCR and ELISA testing. Follow-up whole blood samples were collected in lithium heparin from cats with discordant results and submitted for virus isolation (VI).

Results: There was very good agreement between ELISA and PCR (kappa 0.87; $P < .001$; 95% CI 0.79, 0.95). Of 168 cats, eleven had discordant ELISA/PCR results: 7 ELISA+/PCR- and 4 ELISA-/PCR+. Using VI as a reference standard, there were 4 false-positive PCR results, 5 false-positive ELISA results, and 1 false-negative PCR result (1 cat lost to follow-up).

Conclusions and Clinical Importance: While there was good agreement between the POC ELISA and PCR tests, the discordant results highlight the importance of cautious interpretation of test results and the necessity of confirmatory testing.

Key words: Diagnosis; Infectious disease; Retrovirus.

Feline immunodeficiency virus (FIV) infection causes progressive immune dysfunction, which can result in chronic health problems, neoplasia, or both.[1–4]

Abbreviations:

FeLV	feline leukemia virus
FIV	feline immunodeficiency virus
PCR	polymerase chain reaction
POC	point of care
VI	virus isolation

Infection is most commonly transmitted by penetrating bite wounds, making adult, intact male, outdoor cats at greatest risk of infection due to territorial fighting behavior.[4,5] The overall prevalence of FIV in domestic cats in the United States is approximately 2.5%, with higher prevalence in cats allowed outdoors (4.3%), cats presented to veterinarians with clinical signs of illness (20.6%), and cats in sanctuaries with conditions consistent with animal hoarding (8.8%).[5,6]

The American Association of Feline Practitioners (AAFP) recommends FIV testing for any cat, that is: (1) new to a household, (2) showing signs of clinical illness, (3) FIV-uninfected and living in a high-risk environment (outdoor cats, dynamic household, or living with FIV-infected cats), or (4) FIV-uninfected and recovering from a cat fight.[7] Retesting is recommended at least 60 days after an initial negative test for cats that are new to a household and have a recent history of fighting, or for any kitten initially tested before 6 months of age.[7] Annual testing is recommended for any FIV-uninfected cat living in a high-risk environment.[7]

Initial testing for FIV is typically performed by a point-of-care (POC) ELISA test to detect antibodies against FIV. False-positive results can occur secondary to maternal antibodies in young kittens, or for cats that

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Where the work was done; Sample collection was performed in Chicago, IL at 2 adoption guarantee shelters: PAWS Chicago and Tree House Humane Society and in Memphis, TN, at the Fitzhugh B. Crews FIV Sanctuary, Drennan Animal Hospital, Parkway Village Companion Animal Hospital, and Cordova Station Animal Hospital. The PCR testing was performed at IDEXX Laboratories, West Sacramento, CA. The ELISA testing was performed at IDEXX Laboratories, Westbrook, ME. The virus isolation was performed at the University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. Manuscript preparation and statistical analysis were performed at Purdue University, West Lafayette, IN.

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have received an FIV vaccination.[a,5-8] False-negative results, while less common, can occur before seroconversion, or for cats with end-stage FIV disease in which antibody production can be below a detectable level.[9]

Importantly, cats that received the FIV vaccine are antibody-positive on POC ELISA tests for years after the last FIV vaccination.[8,10,11] The FIV vaccine is not considered a core vaccine and should be used only in high-risk settings.[7] This vaccine is no longer available in North America.

In the case of positive POC ELISA test results for FIV antibodies or high suspicion of disease for a cat with negative results, additional testing is recommended.[7] Virus isolation (VI), considered the reference standard, is not widely available and is expensive and time-consuming to perform. Western blot and immunofluorescent antibody tests for FIV antibodies are available, but sensitivity and specificity are typically lower than those of POC ELISA tests.[9,12] Polymerase chain reaction (PCR) tests amplify FIV nucleic acid in whole blood or other tissue samples. Commercial PCR tests differ by primer set and reaction protocol, and sensitivity and specificity vary.[12,13] The hypothesis tested in this study is that there is substantial agreement between results of a commercially available PCR test for FIV[b] and a POC ELISA test[c] for FIV antibodies for specimens collected from owned and shelter-housed cats.

Methods and Materials

Samples were collected from 168 cats enrolled in an ongoing 5-year longitudinal study following naturally FIV-infected cats. As a condition for enrollment in the 5-year longitudinal study, cats testing positive for feline leukemia virus (FeLV) by POC ELISA[c] tests were excluded from that study, to minimize the confounding effect that more than 1 retroviral infection may have on the health and longevity of the enrolled cats. Cats enrolled in the longitudinal study lived at adoption guarantee shelters (PAWS Chicago, Tree House Humane Society), the Fitzhugh B. Crews FIV Sanctuary, or private homes. For most cats, health and vaccination history before admission to the shelters were unknown. The study protocol was approved by the Purdue University Animal Care and Use Committee (Purdue Animal Care and Use Committee Approval No. 09-011). Health history and vaccination information were updated at each sample collection to include any changes occurring since the last sample collection.

Samples were collected between May 2011 and January 2012. Whole blood samples were collected in K_2-EDTA, divided, and submitted for FIV antibody and FeLV antigen ELISA[c] and polymerase chain reaction (PCR) for FIV[b] as part of the longitudinal study. The limit of detection of the FIV real-time PCR is approximately 600 DNA equivalents per ml of whole blood.[d] The assay tests for both the FIV provirus at the gDNA level and the RNA level (by cDNA after reverse transcription); clinical validation has shown the overall limit of detection is 30 FIV DNA equivalents per ml of whole blood.[d] The diagnostic sensitivity and specificity of the FIV real-time PCR test was in the range of 81–100%,[d] respectively. The clade-specific approach of the FIV real-time PCR used has been adapted from previously published assays and has been tested extensively with isolates from different geographic regions, including Western Europe, North America, and Asia.[13–15] Tested clades include clades A, B, C, D, E, and F.

For any cat with a history of FIV vaccination or discordant POC ELISA and PCR results (positive ELISA/negative PCR or negative ELISA/positive PCR), follow-up testing was performed, including repeating POC ELISA and PCR tests, and shipment of fresh whole blood samples in lithium heparin to the University of Glasgow Centre for Virus Research (Glasgow, United Kingdom) for virus isolation (VI).[16] The follow-up tests were performed between 9 and 16 months (median 10.5 months) after initial testing for cats with negative ELISA/positive PCR results and between 3 and 12 months for cats with positive ELISA/negative PCR results (median 4.5 months).

Data were analyzed by commercially available statistical software.[e] Agreement between ELISA and PCR test results was quantified by kappa and percent concordance. *P*-value and 95% confidence interval were reported for kappa.[e] Discordant results from the initial tests were categorized by VI as the reference standard. Descriptive statistics were also reported.

Results

Of 168 cats sampled, 110 cats were neutered males and 58 were spayed females. The ages of cats ranged from 2 to 15 years with a median age of 5 years for ELISA-positive cats and 4 years for ELISA-negative cats. At the time of sample collection, 49 cats were housed at adoption guarantee shelters, 77 in private homes of 6 cats or fewer, and 42 in the sanctuary or large multicat households of at least 10 cats**.** Four cats were known to have received FIV vaccinations. It was not known whether any of the other 164 cats had received the FIV vaccine.

The initial paired results of ELISA and PCR tests are presented in Table 1. The kappa value was 0.87 ($P < .001$; 95% CI 0.79, 0.95) with 93% concordance, indicating very good agreement between tests.

The 4 cats that had negative ELISA and positive PCR test results subsequently had negative virus isolation results. Sequencing of the products of the PCR tests for these cats revealed that 1 cat had a viral sequence that matched an FIV subtype B sequence, but sequences of the remaining 3 cats' PCR products did not match any reported FIV sequences. All 4 cats with initial negative ELISA/positive PCR results had negative ELISA/negative PCR results at the time of negative VI results.

Of the 7 cats with positive ELISA and negative PCR test results, 1 was positive on VI; 5 had negative VI results; and 1 was lost to follow-up before VI testing. POC ELISA and PCR tests were repeated at the time of VI testing. The 5 cats available for follow-up that had initial positive ELISA/negative PCR results remained ELISA-positive (falsely positive, by VI as the reference standard) and PCR-negative at the time of

Table 1. Comparison of ELISA and PCR results.

		ELISA Result		
		Positive	Negative	Total
PCR Result	Positive	67	4	71
	Negative	7	90	97
Total		74	94	168

PCR, polymerase chain reaction.

negative VI results. The single cat with initial positive ELISA/(false)-negative PCR test results and positive virus isolation had positive ELISA / positive PCR test results at the time of VI-positive results. This cat had persistently positive ELISA and PCR test results through another year of follow-up testing.

The 4 cats with a known history of FIV vaccination all had initial positive ELISA/negative PCR test results. Three had persistently positive ELISA and negative PCR results. One cat had negative ELISA/negative PCR results on follow-up tests. Virus was not isolated from the blood of any of the cats with a known history of FIV vaccination. One of the 4 cats known to have received FIV vaccinations was reported to have received the FIV vaccination 8 years previously, 2 received the vaccine 7 years previously, and for the 4th cat, the date of vaccination was not reported. As part of the longitudinal study, 3 of the vaccinated cats were negative for VI at 3 months and again at 3 years following the test results reported for this study and the 4th cat was negative for VI again 2 years later.

Discussion

Agreement between the results of the POC ELISA and PCR tests used in this study was very good. Using VI as the reference standard, both POC ELISA and PCR tests had similar numbers of false-positive results. This highlights the importance of cautious interpretation of results. However, because only the cats with discordant results were followed with VI, no general statement can be made about proportions of false-positive and false-negative results.

The 4 cats with initial negative ELISA and positive PCR discordant results were FIV-negative on VI and subsequent POC ELISA and PCR tests; however, 1 initial positive PCR discordant sample did match an FIV sequence on viral sequencing. It has been reported that cats living in close contact with seropositive FIV-infected cats can have FIV nucleic acids without developing detectable FIV antibodies or outward clinical signs of infection.[17] It is not known whether seronegative, FIV genome-positive cats will develop active virus production. Cross-contamination of samples for PCR testing cannot be ruled out at either the time of collection or during processing and could provide a possible explanation for the false-positive results of the initial PCR test.

The clinical importance of the discordance between negative ELISA and positive PCR results is uncertain, as it is uncommon for FIV ELISA-negative samples to be submitted for further diagnostics in a routine clinical setting. However, such testing may be performed if a cat is strongly suspected of being infected with FIV, yet has a negative ELISA test result. A negative ELISA test for FIV could occur early in infection before seroconversion or in end-stage infection when antibody production can be below the limits of ELISA detection. If PCR testing is used as an initial screening test, then the risk of false-positive results such as those demonstrated in this study is important. Based on the results of this study, cats that have negative ELISA test results and positive PCR test results should have follow-up PCR, virus isolation testing, or both to confirm FIV infection. In addition, sequencing of PCR products should be requested when such discordance occurs.

There are multiple potential reasons for cats to have positive ELISA test results and negative PCR test results. Viral load fluctuates during FIV infection and in some cats circulating viral load could be too low to allow for amplification during PCR testing.[11,18] This might have been the case for 1 cat in this study that tested positive ELISA, negative PCR initially and later positive ELISA, positive PCR, and VI-positive. Alternative reasons for such discordance could include nucleic acid degradation at the time of sample collection or subsequent handling, or PCR primers that did not detect the virus sequences present**.** For example, worldwide there are 6 subtypes of FIV, identified as A through F, that have been reported.[19–23] Phylogenetic mapping has shown up to 26% genetic diversity between the different subtypes.[21] This diversity can play a role in false-negative PCR results if the primer sequence is optimized to a subtype different than the subtype of infection (ie, PCR primer optimized to subtype A used for a cat infected with subtype F). FIV subtypes A and B are reported from multiple continents; in the United States and Canada, subtypes A, B, C, and F have been reported.[19,20,24] Cats can have positive ELISA test results when not infected with FIV as a result of maternal antibodies (young kittens) or vaccine-induced antibodies, and such cats would have negative PCR test results. Based on the age of cats tested in this study, positive ELISA test results from maternal antibodies can be ruled out as a cause of discordant results for cats that tested ELISA-positive and PCR-negative, as all cats were adults. How long antibodies generated in response to the FIV vaccine persist in cats is not known. Antibodies persist for at least 1 year after vaccination and potentially for 4 or more years.[7] After the initial tests in this study, 1 FIV-vaccinated cat had positive ELISA test results on 2 of 7 tests performed during the larger longitudinal study at 8 and 10 years following the last FIV vaccination, and PCR test results remained negative on 7 subsequent tests. The remaining 3 FIV-vaccinated cats had positive ELISA test results and negative PCR test results on all subsequent tests.

Due to the small sample size of cats known to have received the FIV vaccine, it cannot be determined from this study whether the PCR test used[b] is appropriate to discriminate between cats with positive ELISA test results due to anti-FIV antibody secondary to vaccination, maternal antibody, or FIV infection. A prospective study with a larger number of cats with known FIV vaccination history and FIV infection status would be required to evaluate the utility of this PCR test for such discriminatory purposes.

Although this study shows very good agreement between POC ELISA test[c] results and PCR test[b] results for FIV, there are some important limitations. Infection status was not confirmed by VI in all cats. The samples

for VI were collected between 3 and 16 months after the initial discordant test results were noted. At the time of VI testing, POC ELISA and PCR tests were repeated, and for 5 of the cats, the PCR result had changed (4 changed from positive to negative and 1 from negative to positive), whereas none of the ELISA results had changed. It is not known whether any of the cats in this study were comingled with FIV-positive cats before shelter admission as previous health history and living conditions were unknown. It is possible that the 4 false-positive PCR results were due to the presence of FIV nucleic acids; however, these cats later tested PCR and VI negative. Additionally, it took 2 to 3 days for the samples to be received by the reference laboratory for VI, and this time delay along with potential variation in environmental conditions during shipping could have compromised virus stability and affected the VI result.

In conclusion, there was very good agreement between the POC ELISA and PCR test results for FIV; however, test results must be interpreted with caution as false-positive and false-negative results could occur with each test. Importantly, diagnosis of FIV infection should not be made based on the results of any single test of a sample collected at a single time point. Practitioners should continue to follow current retroviral testing guidelines,[7] and it is further recommended that any discordant POC ELISA and PCR results be followed with repeat POC ELISA and PCR testing, virus isolation, or both if available. In the case of positive PCR and negative ELISA results, request sequencing of the PCR product. The results of this study cannot be generalized to all domestic cats, nor can these results be used to indicate that this PCR test[b] should be used to discriminate between positive ELISA test results because of FIV vaccination or maternal antibody, and positive ELISA test results because of active FIV infection.

Footnotes

[a] Fel-O-Vax FIV®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
[b] FIV RealPCR™ Test, IDEXX Laboratories Inc., Westbrook, ME
[c] SNAP® FIV/FeLV Combo Test, IDEXX Laboratories Inc., Westbrook, ME
[d] IDEXX internal validation data
[e] IBM SPSS Statistics, version 22, IBM Corporation, Armonk, NY

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Conflict of Interest Declaration: Dr. Leutenegger is an employee of IDEXX Laboratories. The conflict of interest was managed in that he did not perform the testing that was done at IDEXX laboratories, and was not involved in the data analysis.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Callanan JJ, Jones BA, Irvine J, et al. Histologic classification and immunophenotype of lymphosarcomas in cats with naturally and experimentally acquired feline immunodeficiency virus infections. Vet Pathol 1996;33:264–272.

2. Hopper CD, Sparkes AH, Gruffydd-Jones TJ, et al. Clinical and laboratory findings in cats infected with feline immunodeficiency virus. Vet Rec 1989;125:341–346.

3. Sparkes AH, Hopper CD, Millard WG, et al. Feline immunodeficiency virus infection. Clinicopathologic findings in 90 naturally occurring cases. J Vet Intern Med 1993;7:85–90.

4. Yamamoto JK, Hansen H, Ho EW, et al. Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. J Am Vet Med Assoc 1989;194: 213–220.

5. Levy JK, Scott HM, Lachtara JL, et al. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. J Am Vet Med Assoc 2006;228:371–376.

6. Polak KC, Levy JK, Crawford PC, et al. Infectious diseases in large-scale cat hoarding investigations. Vet J 2014;201: 189–195.

7. Levy J, Crawford C, Hartmann K, et al. 2008 American Association of Feline Practitioners' feline retrovirus management guidelines. J Feline Med Surg 2008;10:300–316.

8. MacDonald K, Levy JK, Tucker SJ, et al. Effects of passive transfer of immunity on results of diagnostic tests for antibodies against feline immunodeficiency virus in kittens born to vaccinated queens. J Am Vet Med Assoc 2004;225:1554–1557.

9. Barr MC. FIV, FeLV, and FIPV: Interpretation and misinterpretation of serological test results. Semin Vet Med Surg (Small Anim) 1996;11:144–153.

10. Levy JK, Crawford PC, Slater MR. Effect of vaccination against feline immunodeficiency virus on results of serologic testing in cats. J Am Vet Med Assoc 2004;225:1558–1561.

11. Crawford PC, Levy JK. New challenges for the diagnosis of feline immunodeficiency virus infection. Vet Clin North Am Small Anim Pract 2007;37:335–350, vii.

12. Bienzle D, Reggeti F, Wen X, et al. The variability of serological and molecular diagnosis of feline immunodeficiency virus infection. Can Vet J 2004;45:753–757.

13. Crawford PC, Slater MR, Levy JK. Accuracy of polymerase chain reaction assays for diagnosis of feline immunodeficiency virus infection in cats. J Am Vet Med Assoc 2005;226:1503–1507.

14. Leutenegger CM, Klein D, Hofmann-Lehmann R, et al. Rapid feline immunodeficiency virus provirus quantitation by polymerase chain reaction using the TaqMan fluorogenic real-time detection system. J Virol Methods 1999;78:105–116.

15. Pedersen NC, Leutenegger CM, Woo J, et al. Virulence differences between two field isolates of feline

immunodeficiency virus (FIV-APetaluma and FIV-CPGammar) in young adult specific pathogen free cats. Vet Immunol Immunopathol 2001;79:53–67.

16. Hosie MJ, Flynn JN, Rigby MA, et al. DNA vaccination affords significant protection against feline immunodeficiency virus infection without inducing detectable antiviral antibodies. J Virol 1998;72:7310–7319.

17. Dandekar S, Beebe AM, Barlough J, et al. Detection of feline immunodeficiency virus (FIV) nucleic acids in FIV-seronegative cats. J Virol 1992;66:4040–4049.

18. Beebe AM, Dua N, Faith TG, et al. Primary stage of feline immunodeficiency virus infection: Viral dissemination and cellular targets. J Virol 1994;68:3080–3091.

19. Phadke AP, de la Concha-Bermejillo A, Wolf AM, et al. Pathogenesis of a Texas feline immunodeficiency virus isolate: An emerging subtype of clade B. Vet Microbiol 2006; 115:64–76.

20. Weaver EA. A detailed phylogenetic analysis of FIV in the United States. PLoS ONE 2010;5:e12004.

21. Sodora DL, Shpaer EG, Kitchell BE, et al. Identification of three feline immunodeficiency virus (FIV) env gene subtypes and comparison of the FIV and human immunodeficiency virus type 1 evolutionary patterns. J Virol 1994;68:2230–2238.

22. Bachmann MH, Mathiason-Dubard C, Learn GH, et al. Genetic diversity of feline immunodeficiency virus: Dual infection, recombination, and distinct evolutionary rates among envelope sequence clades. J Virol 1997;71:4241–4253.

23. Kakinuma S, Motokawa K, Hohdatsu T, et al. Nucleotide sequence of feline immunodeficiency virus: Classification of Japanese isolates into two subtypes which are distinct from non-Japanese subtypes. J Virol 1995;69:3639–3646.

24. Reggeti F, Bienzle D. Feline immunodeficiency virus subtypes A, B and C and intersubtype recombinants in Ontario, Canada. J Gen Virol 2004;85:1843–1852.